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ORIGINAL ARTICLE

Common melanocortin-3 receptor variants are not associated with obesity, although rs3746619 does influence weight in obese individuals

Doreen Zegers · Sigri Beckers · Ilse L. Mertens · Luc F. Van Gaal · Wim Van Hul

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Abstract The melanocortin-3 receptor is a vital link in the leptin–melanocortin signaling pathway in the brain and has a role in the regulation of energy homeostasis. It was hypothesized that common polymorphisms in *MC3R* could increase susceptibility for the development of obesity, but different studies have led to contradictory results. In this study, we investigated the association of SNPs in *MC3R* with the development of obesity in an extensive Caucasian population. Using the HapMap, we selected two tagSNPs (rs6127698 and rs3746619) that cover all of the common genetic variation in *MC3R* and genotyped them in 1008 obese cases and 313 normal weight controls. Statistical analysis of the data showed that none of the analyzed SNPs were associated with obesity. However, linear regression analysis did show that SNP rs3746619 has an influence on weight ($P = 0.015$) in the obese population only. Furthermore, a trend for association with BMI in the obese population was observed for this SNP ($P = 0.039$). Taken together, these data are consistent with the involvement of rs3746619 in weight regulation among obese individuals. However, further research including replication of our results is necessary to elucidate the role of *MC3R* in complex obesity.

Keywords MC3R · Obesity · Genetics · Association study

D. Zegers · S. Beckers · W. Van Hul (✉)
Department of Medical Genetics, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium
e-mail: wim.vanhul@ua.ac.be

I. L. Mertens · L. F. Van Gaal
Department of Endocrinology, Diabetology and Metabolism, Antwerp University Hospital, Antwerp, Belgium

Introduction

The melancortin-3 receptor (MC3R) belongs to the family of seven transmembrane (7TM) G-protein coupled melanocortin receptors (MCRs) [1]. Together with the well-known melanocortin-4 receptor (MC4R), MC3R is mainly expressed in the brain. The seven transmembrane (7TM) receptor is predominantly expressed in several nuclei of the hypothalamus that are involved in regulating energy metabolism [1–3] and plays an important role in the leptin–melanocortin signaling pathway in the brain that regulates energy metabolism. By studying Mc3r and Mc4r deficient mice, the role of both MC3R and MC4R receptors in energy homeostasis was demonstrated [4, 5]. It was shown that $Mc3r^{-/-}$ mice display an increase in adipose mass of ~50–60% and a higher feed efficiency compared to wild-type (WT) littermates, though they are hypophagic and maintain a normal metabolism. It is, therefore, suggested that in the absence of *Mc3r*, nutrients are preferentially partitioned into fat mass [5, 6]. This indicates that the MC3R receptor is involved in the regulation of energy metabolism by regulating the balance between fat mass and lean mass. Furthermore, MC3R receptors are suggested to be important for adapting metabolism and maintaining circadian rhythms during periods of limited food availability [7, 8]. This all demonstrates that MC3R is a very important receptor, which is involved not only in weight regulation, but also in more complex mechanisms that influence energy homeostasis.

Hypothesizing that MC3R defects, similar to most MC4R defects, could be the cause of dominantly inherited obesity, mutation screening of the gene was performed in large cohorts of obese individuals. Although several mutations in *MC3R* have been identified [9–13], the disease causality of these mutations remains controversial [13].

Furthermore, the occurrence of rare variants in *MC3R* in a cohort of lean controls has also been reported, suggesting that not all *MC3R* mutations cause severe obesity [12]. A second hypothesis states that *MC3R* could be involved in complex obesity, where common polymorphisms in the gene act together with environmental factors to increase susceptibility for the development of obesity. As positive linkage was found for human chromosome 20q13 in a genome scan for obesity, genes in this region became good candidates for further genetic research in order to explain the heritability of obesity [14]. The *MC3R* gene, which maps to 20q13.2–q13.3, is therefore an excellent candidate gene for obesity research. Several research groups attempted to investigate association of variants in *MC3R* with obesity, but limited number of study subjects, ethnically diverse study populations and a variety of genotyping techniques led to contradictory results [10, 15–18]. In this study, we covered the entire variation in a 2 kb region around the *MC3R* gene. By genotyping a large sample set of obese patients and normal weight control subjects, all from Caucasian origin, and using very robust genotyping assays, we were able to further evaluate the contribution of *MC3R* variants to the pathogenesis of obesity.

Results

Single nucleotide polymorphism (SNP) selection was based on the data provided by the HapMap project (Data Rel 24). After downloading the data into Haploview and running the program, the output showed that genotyping of 2 tagSNPs (rs6127698 and rs3746619) was sufficient to capture all informative SNPs (MAF $\geq$ 5%) in the selected region with $r^2 > 0.8$. Genotypes for rs6127698 and rs3746619 were obtained for 1008 obese patients and 313 normal weight controls (Allele frequencies: Table 1). The genotype distribution of both polymorphisms was in Hardy–Weinberg equilibrium ($P > 0.05$). We compared genotype frequencies of the selected SNPs between cases and controls using chi square, where none of the analyzed SNPs were found to be associated with obesity (Table 1). By performing logistic regression, we confirmed that neither rs3746619 (OR = 1.009, 95% CI = 0.710–1.434, $P = 0.958$) nor rs6127698 (OR = 0.917, 95% CI = 0.759–1.107, $P = 0.365$) is associated with obesity. We also performed linear regression analysis in order to measure the effect of the *MC3R* variants on obesity-related parameters. Here, we found that in our entire population rs3746619 was not associated with body mass index (BMI), weight, waist, waist-to-hip ratio (WHR), total fat area (TFA), visceral fat area (VFA), or subcutaneous fat area (SFA) (all P values > 0.05, data not shown). However, we did find rs3746619 to be associated with weight as a quantitative trait ($P = 0.015$, adjusted for age and sex) in the obese population ($N = 1008$) (Table 2). Furthermore, a clear trend for association of this SNP with BMI ($P = 0.039$, adjusted for age and sex) was observed. The P value for this association with BMI does not withstand the Bonferroni correction for multiple testing ($P < 0.05/2 = 0.025$). Conversely, the association with weight ($P = 0.015$) still stands after correcting for the analysis of two independent TagSNPs. SNP rs6127698 was not associated with any obesity-related parameter ($P > 0.05$, data not shown).

Table 1 Genotype frequencies of tagSNPs in cases and controls

Cases		Controls	*P* value
rs6127698			
TT	240 (23.8%)	66 (21.1%)	0.568
TG	510 (50.6%)	161 (51.4%)	
GG	258 (25.6%)	86 (27.5%)	
rs3746619			
AA	6 (0.6%)	2 (0.6%)	0.991
AC	136 (13.5%)	43 (13.7%)	
CC	866 (85.9%)	268 (85.6%)	

Nominal *P* values are given for Pearson chi square analysis

Discussion

In this study, we could not assess a clear association between common polymorphisms in or near the *MC3R* gene and obesity in a case–control design. However, when we analyzed quantitative obesity-related parameters, we did find rs3746619 to influence weight in the obese population and found a trend for association with BMI. Both the genotyped SNP rs3746619 and SNP rs3827103, which is in complete linkage disequilibrium (LD) with the previous one, are coding polymorphisms within the *MC3R* gene. They, respectively, represent amino acid substitutions T6K and V81I and are obvious SNPs to genotype.

Multiple association studies have already been performed with variants in and near *MC3R*. To our knowledge, this is the first study that assayed the entire variation in a 2 kb region surrounding the *MC3R* gene in an extensive homogeneous Caucasian population. In the study by Li et al. no significant allele differences were found for the coding SNP rs3827103 (V81I) and several other polymorphisms in the 5′ region of the gene [15]. Both the obese ($N = 124$) and control group ($N = 85$) contained Caucasians as well as African-Americans, which makes comparison with our study population difficult. Boucher et al. reported the association of the +2138InsCAGACC polymorphism, a 6 base pair insertion located 2138 base pairs

Table 2 Associations of obesity-related parameters and genotype of rs3746619 in obese individuals

Mean ± SEM		*P* value
BMI		
CC	38.0 ± 0.2	0.039
CA	38.9 ± 0.5	
AA	41.0 ± 3.3	
Weight (kg)		
CC	110.8 ± 0.7	**0.015**
CA	114.2 ± 1.9	
AA	118.6 ± 15.0	
Waist (cm)		
CC	116.0 ± 0.5	0.172
CA	116.7 ± 1.3	
AA	120.8 ± 10.8	
WHR		
CC	1.00 ± 0.01	0.684
CA	0.99 ± 0.01	
AA	1.00 ± 0.01	
TFA (cm^2)		
CC	749 ± 6	0.185
CA	768 ± 14	
AA	781 ± 87	
VFA (cm^2)		
CC	183 ± 3	0.825
CA	178 ± 7	
AA	182 ± 41	
SFA (cm^2)		
CC	566 ± 5	0.089
CA	590 ± 13	
AA	599 ± 85	

Mean value ± standard error of the mean (SEM) is given. *P* values, adjusted for age and sex, are derived from linear regression analysis of the data. *P* values shown in bold withstand the Bonferroni-correction for multiple testing ($P < 0.05/2 = 0.025$)

WHR waist-to-hip ratio; *TFA* total fat area; *VFA* visceral fat area and *SFA* subcutaneous fat area as measured by CT-scan

downstream of the *MC3R* start codon, and body composition related phenotypes, while again no association between V81I (rs3827103) and body composition was found. However, as the obesity-related variables BMI and body weight were not analyzed, it is difficult to compare their study with our results [16]. Although Schälin-Jantti et al. also detected the rs6127698 (−373G>T) variant 5′ of the *MC3R* gene, they did not genotype this SNP in their entire population, which makes comparison with our study impossible. They also assessed the genotype frequencies of T6K (rs3746619), V81I (rs3827103), and −239A>G (rs11575886, a variant in the putative promoter region of *MC3R*) and concluded that they did not differ between obese cases ($N = 244$) and controls ($N = 225$) [17]. More recently, Lee et al. also genotyped the coding SNPs in *MC3R* and found no difference in genotype frequencies in controls ($N = 83$) compared to the obese group ($N = 198$), although they did describe obese subjects carrying 6K/81I to have a higher percentage of bodyfat [10]. Another interesting study by Savastano et al. describes a positive association between the T6K (rs3746619) and V81I (rs3827103) polymorphisms and pediatric obesity which is probably due to greater energy intake. They found that body mass index and fat mass were elevated in children with double homozygosity for both SNPs [18]. As the study population only includes children aged 6–19 years, it is difficult to draw conclusions toward the influence of these polymorphisms on adult obesity. In the present study, we found rs3746619 to quantitatively influence weight in the obese population, resulting in increased weight in obese individuals carrying the 6K/81I genotype (Table 2). Even though we did not observe allele frequency differences between obese subjects and normal weight controls and could not identify rs3746619 as a SNP that increases susceptibility for the development of obesity, we were able to pick up subtle effects on weight of obese individuals caused by this SNP. Possibly, our study does not have enough power to pick up a rather small effect in a case–control analysis. This could also explain why none of the *MC3R* SNPs were reported as top hits in genome-wide association studies. We pick up an association with SNP rs3746619 that is consistent with an additive model (Table 2). However, as the minor allele frequency (MAF) of rs3746619 is only 0.073 and the number of AA homozygote subjects is small, caution must be called in interpreting the results and larger replication studies are needed to confirm our findings.

Our results are not in line with most other *MC3R* association studies, where in adult populations no effect for rs3746619 is reported. A possible explanation for this discrepancy is the variety of different ethnicities in the study populations used. The allele frequencies of the tagged SNP rs3746619 and SNP rs3827103 vary considerably between populations, with a minor allele frequency of only 0.08 in the Caucasian population and up to 0.58 in the African population (HapMap Data Rel 24/phaseII Nov08, on NCBI B36 assembly, dbSNP b126). Furthermore, our study population is ethnically homogeneous and quite large, which may enable us to pick up smaller and more subtle effects. In addition, the involvement of rs3746619 and rs3827103 in the pathogenesis of obesity was corroborated by in vitro experiments on 6K/81I *MC3R* expressing cells, indicating a functional effect of the polymorphisms [19].

Although these data are consistent with the involvement of a common coding SNP in *MC3R* in weight regulation among obese individuals, we cannot exclude that

polymorphisms with a lower minor allele frequency or rare variants in *MC3R* are associated with the pathogenesis of obesity, because we lack power to detect the effects caused by these variants. Furthermore, it is also possible that SNPs further upstream or downstream of the gene have an additional influence on the development of the disease. In addition to replication of our results in even bigger study populations or meta-analyses, these hypotheses should be the focus in future studies investigating the role of *MC3R* in complex obesity.

Materials and methods

Study population

One thousand and eight obese individuals (468 men and 540 women) were recruited from the outpatient obesity clinic at the Antwerp University Hospital. Only patients with a body mass index (BMI) of 30 or more and age above 20 years were included in the study. Patients with diabetes or impaired glucose tolerance as well as postmenopausal women were excluded from the study. Three hundred and thirteen normal weight control subjects (BMI $\leq$ 25 kg/m^2) were recruited among employees from the university and the university hospital and among couples seeking prenatal counselling at the Department of Medical Genetics. All study subjects are of Belgian Caucasian origin. Population characteristics of cases and controls are summarized in Table 3. The study was approved by the local ethics committee and all participants gave their written informed consent once the aim and design of the study had been explained. Using this study population, we have 80% power to detect association of a SNP with a minor allele frequency of 0.30 and a relative risk of 1.3. For all patients and controls, height was measured to the nearest 0.5 cm and body weight was measured with a digital scale to the nearest 0.1 kg. BMI was calculated as weight (in kg) over height (in m) squared. Waist circumference was measured at mid-level between the lower rib margin and the iliac crest, and hip circumference at the level of the trochanter major. Total fat area (TFA), visceral fat area (VFA), and subcutaneous fat area (SFA) were determined with CT-scan at the L4-L5 level [20].

Table 3 Characteristics of the cohort obese adults and normal weight control subjects

Parameter	Obese cases	Control subjects
N	1008	313
Age (years)	41.6 ± 0.4	35.3 ± 0.4
Men/women	468/540	98/215
Weight (kg)	111.3 ± 0.7	65.2 ± 0.5
Height (m)	1.71 ± 0.01	1.71 ± 0.01
BMI (kg/m^2)	38.1 ± 0.2	22.1 ± 0.1
Waist (cm)	116.2 ± 0.5	n/a
WHR	1.00 ± 0.01	n/a
TFA (cm^2)	751 ± 5	n/a
VFA (cm^2)	183 ± 3	n/a
SFA (cm^2)	570 ± 5	n/a

Mean value ± standard error of mean is given

WHR waist-to-hip ratio; *TFA* total fat area; *VFA* visceral fat area and *SFA* subcutaneous fat area as measured by CT-scan

Genotyping

Blood samples from all the patients and control subjects were obtained for extraction of genomic DNA by standard procedures [21].

For our association study, we used the presence of linkage disequilibrium (LD) between SNPs to reduce the number of SNPs needed to cover all variation in the gene. A region of 2 kb surrounding the *MC3R* gene was selected (HapMap Data Rel 24/phaseII Nov08, on NCBI B36 assembly, dbSNP b126; chr20:54256737–54258736) and SNP genotyping data from the CEPH population was downloaded into Haploview [22] in order to select the appropriate tagSNPs. Genotypes of SNPs were determined using TaqMan SNP Genotyping assays (ABI, Foster City, USA). Assays were performed according to instructions by the kit manufacturers and analysis of TaqMan assays was done on a LightCycler® 480 Real-Time PCR System (Roche Applied Science, Mannheim, Germany). Blank samples and samples with known genotype were included as negative and positive controls, respectively.

Statistics

For each SNP, Hardy–Weinberg equilibrium (HWE) was calculated using the De Finetti program (Thomas F. Wienker, http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). Genotype and allele frequencies among cases and controls were compared using Pearson's chi square analysis and odds ratios for both variants were calculated using logistic regression (adjusted for age and sex). Significance level was set at $P = 0.05$. Linear regression, also adjusted for age and sex, was used to investigate whether the variants have an influence on BMI or weight, in order to model the relationship between obesity-related parameters and the genotype of a SNP. Again, significance level was set at $P = 0.05$. All statistical analyses were performed using SPSS version 15.0 (SPSS, Chicago, IL, USA). The program Quanto (http://hydra.usc.edu/GxE/) was used for power calculations.

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